THE BUDDING AND DEPTH OF INVASION -MALLI SUUSYÖVÄSSÄ – SYSTEMAATTINEN KATSAUS JA META-ANALYYSI

Onkamo, Oona

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TIIVISTELMÄ

Onkamo, Oona: The Budding and Depth of Invasion -malli suusyövässä – systemaattinen katsaus ja meta-analyysi

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Suun levyepiteelikarsinooma on sairaus, jonka ennuste on suhteellisen huono. Sillä on myös korkea uusiutumistaipumus. Vuonna 2015 esitellyn BD-mallin avulla taudin etenemistä voidaan mahdollisesti ennustaa perinteisiä menetelmiä tarkemmin. BD-mallin nimi tulee englanninkielen sanoista budding ja depth. Se koostuu kahdesta parametristä, joita ovat syöpäsolujen muodostamien nuppujen lukumäärä (budding) sekä kasvaimen invaasiosyvyys (depth). Tässä tutkimuksessa tutkimuskysymyksenä oli ”onko BD-mallilla prognostista arvoa suun levyepiteelikarsinoomaa sairastavien potilaiden hoidossa?” Aineistona oli vuoden 2020 helmikuuhun mennessä julkaistut tutkimukset, joissa käsiteltiin suusyöpää ja BD-mallia. Tutkimusaineisto kerättiin tekemällä systemaattinen haku neljästä eri tietokannasta: Pubmedistä, Scopuksesta, Ovid Medlinesta ja Web of Sciencesta. Systemaattisen haun suorittivat kaksi kirjoittajaa itsenäisesti, ja myöhemmin hakujen tulokset yhdistettiin yhteiseksi tutkimusaineistoksi. Hakuehdot systemaattiseen katsaukseen täytti yhdeksän tutkimusta, joista neljä otettiin mukaan myös meta-analyysiin. Tutkimuksemme tulokset osoittivat, että BD-mallilla on prognostista arvoa suun levyepiteelikarsinooman diagnostiikassa ja hoidossa. BD-mallin avulla voidaan myös arvioida potilaiden ennustetta sekä taudista selviytymistä.

Avainsanat: diagnostiikka, levyepiteelikarsinooma, meta-analyysi, suusyöpä, systemaattinen katsaus

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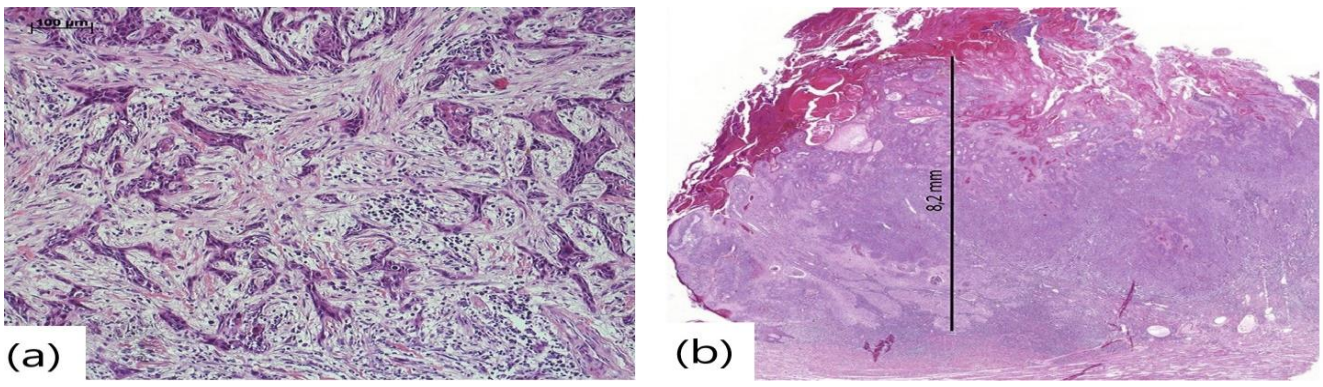
LIITTEET

Liite 1. The budding and depth of invasion model in oral cancer: A systematic review and meta-analysis -artikkeli

1. JOHDANTO

Suun levyepiteelikarsinooma (OSCC) käsittää lähteestä riippuen 4,7% - 20,3% kaikista pään ja kaulan alueen syövistä (Chen ym. 2016; Siegel ym. 2017). Potilaiden ennuste on suhteellisen huono: alle puolet potilaista on elossa 5 vuotta taudin diagnosoinnin jälkeen (Kim ym. 2016). Uusiutuneen OSCC:n saaneilla potilailla 20-40% todetaan kaulan imusolmukemetastaasi (Bittar ym. 2016; Safi ym. 2017). WHO:n vuonna 2017 julkaiseman kirjan mukaan OSCC:n hoitolinjan valinta perustuu kasvaimen cTNM-luokitteluun sekä histologiseen diagnoosiin (El-Naggar ym. 2017). OSCC:n ennusteeseen ja hoitovasteeseen vaikuttavat kuitenkin myös muut tekijät (Almangush ym. 2015). Almangush kumppaneineen esitteli BD-mallin, minkä avulla voidaan OSCC:n etenemistä voidaan ennustaa ja täten myös suunnitella sen hoitoa tarkemmin.

BD-malli koostuu kahdesta histologisesta parametrista, jotka ovat kasvaimen budding (”nuputtautuminen”, B) ja kasvaimen invaasiosyvyys (D). Kasvaimen nuputtautumisella tarkoitetaan alle 5 syöpäsolun muodostamaa solurypästä kasvaimen invasiivisella puolella. Nuputtautumista kuvaa kuvan 1 a-kohta. Runsas nuputtautuminen on yhteydessä esimerkiksi kohonneeseen riskiin kehittää kaulan imusolmukemetastaasi. Kasvaimen invaasiosyvyydellä tarkoitetaan mittaa epiteelin basaalisolukosta kasvainsolukon syvimpään pisteeseen ja sitä on havainnollistettu kuvan 1 b-kohdassa. (Almangush ym. 2015)



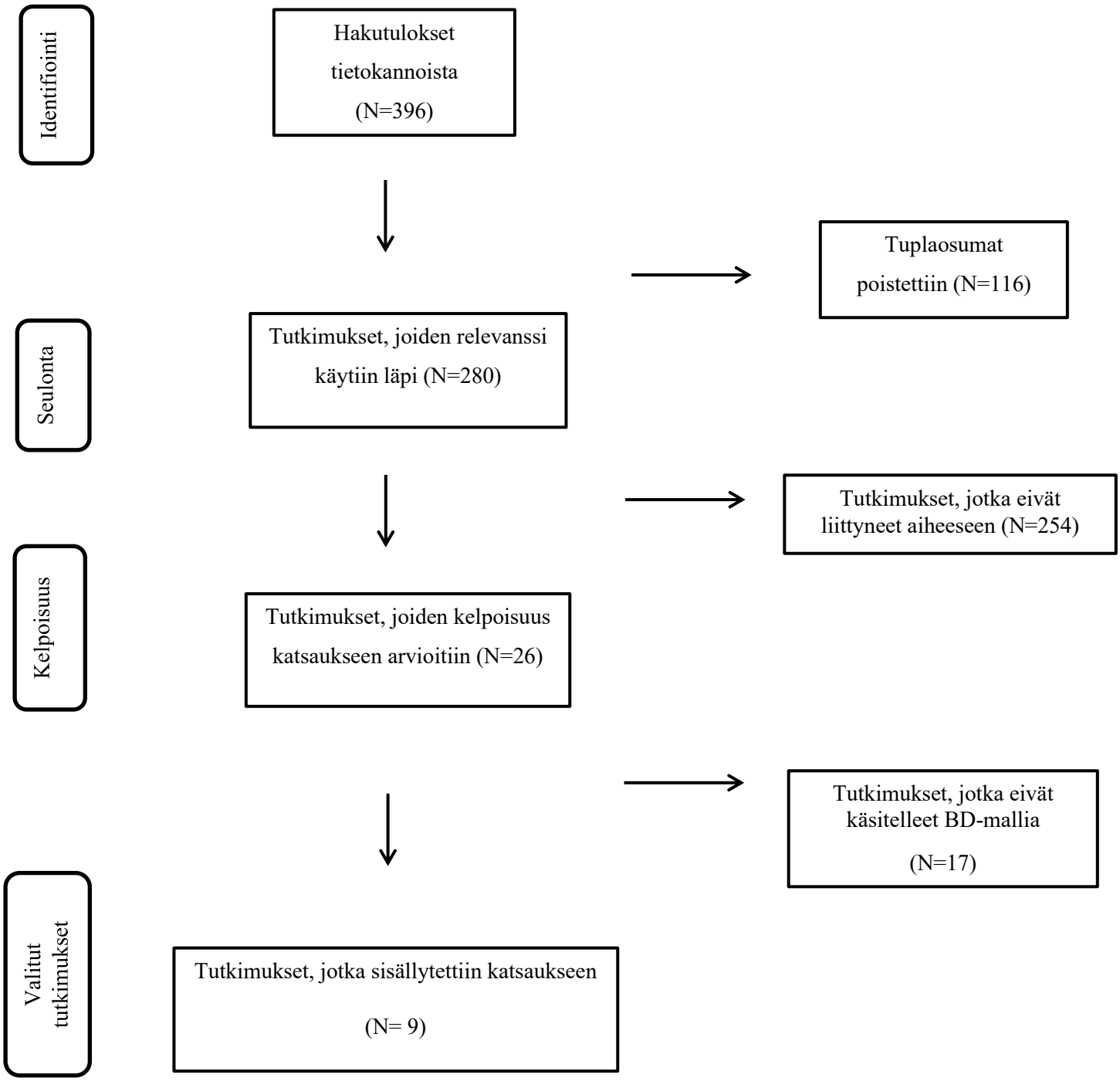
Kuva 1. a) Kasvaimen muodostamia soluryppäitä, joissa kussakin alle 5 kasvainsolua, b) Invaasiosyvyys epiteelin basaalisolukosta kasvaimen syvimpään kohtaan.

BD-mallin prognostista arvoa on tutkittu useissa tutkimuksissa käsittäen suuontelon eri osia. Tulokset ovat tukeneet BD-mallin potentiaalia itsenäisenä prognostisena mittarina.

2. MENETELMÄT

Aloitimme systemaattisen katsauksen tekemällä systemaattisen haun artikkelin ensimmäisen kirjoittajan kanssa, molemmat itsenäisesti ja toisistamme riippumattomasti. Etsimme tutkimuksia PubMedista, OvidMedlinesta, Scopuksesta ja Web of Sciencesta hakusanoilla (oral OR mouth OR tongue OR floor of the mouth OR lip OR gingiva OR buccal OR palate) AND (cancer OR carcinoma OR neoplasm OR tumor OR tumour) AND (bd OR bd model OR budding and depth OR budding and depth of invasion). Itse sain tähän apua Oulun yliopiston lääketieteellisen tiedekunnan kirjaston informaatikolta Margit Heikkalalta. Kävimme ensimmäisen kirjoittajan kanssa ensin itsenäisesti läpi tuloksiamme ja sitten vertailimme niitä toistemme Refworks-tilien kautta. Tutkimuskysymyksenä oli ”onko BD-mallilla prognostista arvoa OSCC-potilaiden hoidossa?” Suljimme pois tutkimukset, joita ei ollut kirjoitettu englanniksi, joissa tutkittiin eläimiä ja joissa ei tutkittu BD-mallia. Suljimme pois myös konferenssitiivistelmät. Täytimme molemmat tahoillamme Prisma Flow -taulukon (katso taulukko 1) ja varmistimme, että lukuarvot ovat molemmilla samat.

Taulukko 1. Hakutulosten haku tietokannoista ja katsaukseen valitut tutkimukset.



Kasvaimen nuputtautumiselle (B) ja invaasiosyvyydelle (D) annettiin numeroarvot, jolloin BD-malli sai lukuarvot 0, 1 tai 2. Matalaa riskiä (0) ennustavat alle 4 mm invaasiosyvyys ja alle 5 soluryvästä kasvaimen invasoivalla puolella. Keskitason riskiä (1) ennustavat 4 mm tai yli invaasiosyvyys ja alle 5 soluryvästä, tai alle 4 mm invaasiosyvyys ja 5 tai useampi solurypäs. Korkeaa riskiä (2) ennustavat yli 4 mm invaasiosyvyys ja 5 tai useampi solurypäs.

3. TULOKSET

3.1. Tulokset

Tietokannoista löytyi edellä mainituilla hakukriteereillä 396 osumaa, joista 116 oli tuplaosumia. Täten 280 tutkimusta sisällytettiin jatkoseulontaan. Jokainen näistä artikkeleista käytiin manuaalisesti läpi, sekä minä että artikkelimme ensimmäinen kirjoittaja, aluksi itsenäisesti ja sitten tuloksiamme vertaillen. Tutkimuksista 9 käsitteli BD-mallia OSCC:ssa. Nämä 9 tutkimusta sisällytettiin systemaattiseen katsaukseen. Näistä tutkimuksista 3 käsittelee japanilaisia, 1 suomalaisia, 2 suomalaisia ja brasilialaisia, 2 brasilialaisia ja 1 kiinalaisia potilaita. Tutkimusten tulokset on koottu taulukkoon 2. Allekirjoittanut täytti aluksi 4 saraketta ja ensimmäinen kirjoittaja 5. Tämän jälkeen tarkistimme toistemme sarakkeet ja teimme tarvittavat tarkennukset/ korjaukset.

Taulukko 2. Katsaukseen valittujen tutkimusten tulokset.

Authors, year (Country)	No. of cases	TNM stage	Oral subsite	Primary treatment	Follow-up	Staining method	Cutoff for budding	Cutoff for depth	Survival Analysis/ Sensitivity, Specificity	HR (95% CI)	*P*-value	REMARK Quality of studies
Almangush *et al*, 2015 (Finland and Brazil)	311	T1-2N0	Tongue	Surgery	57 months	H-E	5 buds	4 mm	DSS	6.24 (2.59-15.04) **5.11 (2.05-12.75)**	< 0.001 **< 0.001**	7
									DFS	2.14 (1.22-3.74) **2.19 (1.20-4.00)**	0.025 **0.033**	
Sawazaki-Calone *et al,* 2015 **(**Brazil**)**	113	T1-T4, N0, N+	Oral cavity	Surgery, surgery + postoperative RT, surgery + postoperative RT+CT	5 years	H-E	5 buds	4 mm	DSS	DSS in 5 yrs 32%	0.009	6
									DFS	**1.93 (1.23-3.00)** DFS in 5 yrs 49%	**0.003** 0.005	
									HRS		<0.001	
Seki *et al*, 2016 **(**Japan**)**	91	T1-T4	Tongue and floor of mouth	Surgery, preoperative CT	4 months – 5 years	IHC	≥3 buds	≥3 mm	OS		< 0.05	6
									DFS		**< 0.05**	
									Sensitivity	100% sensitivity ≥3mm budding		

Strieder *et al,* 2017 **(**Brazil**)**	53	T1-T4, N0,N+	Lip	Surgery, surgery + RT	57.5 months (T1/T2), 159.4 months (T3/T4)	H-E	5 buds	4 mm	OS (5 years)	75% (high risk cases)	0.045	6
Hori *et al,* 2017 **(**Japan**)**	48	cT1/2 N0M0	Tongue	Surgery	71 months	H-E	5 buds	≥3 mm	Sensitivity Specificity	89% Sensitivity 95% Specificity 80% PPV 97% NPV		6
Almangush *et al,* 2018a. **(**Finland and Brazil**)**	224	T1-2N0	Tongue	Surgery	NA	H-E	5 buds	4mm	DFS DSS	**3.42 (1.71-6.82)** 2.82 (1.46-5.42) **11.63 (3.83–35.31)** 10.43 (3.51-31.01)	**0.004** 0.014 **< 0.001** < 0.001	8
Almangush *et al*, 2018b **(**Finland**)**	100	NA	Tongue	Surgery	NA	H-E	5 buds	4mm	Sensitivity Specificity	57.1% (95% CI 39.4 - 73.7%) 96.9% (95% CI 89.3-99.6%)	Chi-square test **<** 0.001	6
Yu *et al,* 2019 **(**China**)**	246	T1- T2/ T3-T4	Tongue	Surgery	60 months	H-E	5 buds	4 mm	OS DFS	BD, 2.77 (1.78-4.33) BD, 1.66 (1.21-2.27)	< 0.001 0.002	8

Hori *et al,* 2020 **(**Japan**)**	62	T1-2N0	Tongue	Surgery	68 months	H-E and PCK	5 buds	≥3 mm	DFS Lymph node recurrence	**BD, 2.06 (0.64-6.57)** BD, 5.46 (1.08-27.52)	0.22 < 0.05	7

3.2 Disease-free survival: meta-analyysi

Ainoastaan 4 tutkimusta osoitti tarkan tilastollisen analyysin koskien disease-free survivalia (DFS eli tauditonta hoidon jälkeistä jaksoa), sisältäen HR-arvot ja 95% luottamusvälin. Nämä 4 tutkimusta sisällytettiin meta-analyysiin. Ensimmäisessä vaiheessa käsiteltiin kaikki monimuuttuja-analyysin tulokset, joissa tutkittiin BD-mallin prognostista kykyä ja yhteyttä DFS:iin. Toisessa vaiheessa yhdistettiin monimuuttuja-analyysin ja yhden muuttujan analyysin tulokset, jolloin saatiin kumulatiivinen otanta (cumulative effect size). Tutkimuksissa todettiin tautispesifin selviytymisen (disease-specific survival =DSS) ja kokonaisselviytymisen (overall survival =OS) yhteys BD-malliin. Eräässä katsauksen tutkimuksessa (Sawazaki-Calone et al 2015), jossa olivat mukana perinteiset prognostiset mallit (WHO:n tuumoriluokittelu, histologinen riskimalli, HR), ainoastaan BD-mallilla oli yhteys DSS:iin. Myös OS:lla ja BD-mallilla oli tilastollisesti merkitsevä korrelaatio useassa tutkimuksessa (Seki et al 2016, Strieder et al 2017, Yu et al 2019).

3.3 BD-mallin yhteys kliinispatologisiin tekijöihin

Yhteys BD-mallin ja HR-mallin välillä raportoitiin useassa tutkimuksessa. Kasvaimen nuputtaumisella ja invaasiosyvyydellä havaittiin tilastollisesti merkitsevä yhteys kaulan metastaaseihin. Myös syövän leviäminen kaulan imusolmukkeisiin oli yhteydessä BD-malliin.

4. POHDINTA

Tutkimuksen tavoitteena oli osoittaa BD-mallin prognostinen merkitys OSCC:ssa. Vuoden 2017 WHO classification of head and neck tumours osoitti, että nykyinen histologiseen diagnoosiin perustuva (hyvin, kohtalaisesti tai heikosti erilaistunut OSCC) luokittelu pään ja kaulan alueen kasvaimissa korreloi huonosti hoidon lopputuloksen kanssa. Kasvaimen histologiaa on helppo analysoida ja se on siksi yhä laajassa käytössä, vaikka sitä on kritisoitu (Brierley ym. 2017). Tekemämme meta-analyysi osoittaa BD-mallin olevan hyvä prognostinen työkalu, jolla on merkitsevä yhteys DFS:iin. Useissa tutkimuksissa myös useat kliinispatologiset tekijät (kuten imusolmukemetastaasit ja sairauden kliininen vaihe) olivat merkitsevässä yhteydessä BD-malliin.

Systemaattiseen katsaukseen otetuista tutkimuksista yksi osoitti merkitsevän korrelaation BD-mallin ja OS:n välillä (Yu ym. 2019). Tämä tutkimus ja Almagushin tutkimus vuodelta 2015 osoittivat merkitsevän yhteyden myös BD-mallin DSS:n välillä. BD-mallin tarkkuus tutkimuksissa oli 93% ja 96,9% (Hori ym. 2017 ja Almangush ym. 2018). Nämä tutkimukset olivat kuitenkin puutteellisia, eikä niitä voitu sisällyttää meta-analyysiin OS:n ja DSS:n osalta.

Kaikissa systemaattisen katsauksen tutkimuksista ensisijaisena hoitona OSCC:iin oli leikkaus. Suurin osa tutkimuksista käsitteli kielen levyepiteelikarsinoomaa (OTSCC). Sisällytimme meta-analyysiin myös muualla suuontelossa esiintyviä levyepiteelikarsinoomia. Eri tutkimuksissa sairaudet olivat eri vaiheissa. Nämä ovat tutkimuksemme rajoitteita.

Soluryppäiden määrän raja-arvona korkeaan riskiin pidettiin useimmissa tutkimuksissa 5 rypästä, mutta yksi tutkimusryhmä (Seki ym. 2016) asetti raja-arvoksi 3 solurypästä. Soluryppäät edustavat aktiivista invaasiota ja solujen epäjärjestystä. Sillä on todettu yhteys huonoon ennusteeseen myös esimerkiksi rinta- ja maksasyövässä.

Invaasiosyvyydellä on yhteys imusolmukemetastaaseihin ja syövän uusiutumiseen. 6 tutkimusta asetti invaasiosyvyyden raja-arvoksi 4 mm, japanilaistutkimuksissa raja-arvo oli 3 mm. Invaasiosyvyyden lisääminen TNM-luokitteluun parantaisi OSCC:n ennustettavuutta.

BD-malli on suhteellisen yksinkertainen ja objektiivinen tapa arvioida kasvaimen luonnetta. Se on myös kustannustehokas ja helposti lisättävissä tällä hetkellä käytössä olevaan histopatologiseen tutkimukseen.

5. JOHTOPÄÄTÖKSET

Systemaattinen katsauksemme osoittaa, että BD-mallilla on vahva kyky ennustaa OSCC-potilaiden selviytymistä.

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LIITTEET

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REVIEW ARTICLE

ORAL DISEASES WILEY

The budding and depth of invasion model in oral cancer: A systematic review and meta-analysis

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Abstract

Background: Tumour budding (B) and depth of invasion (D) have both been reported as promising prognostic markers in oral squamous cell carcinoma (OSCC). This meta-analysis assessed the prognostic value of the tumour budding and depth of invasion combination (BD model) in OSCC.

Methods: Databases including Ovid MEDLINE, PubMed, Scopus and Web of Science were searched for articles that studied the BD model as a prognosticator in OSCC. PICO search strategy was "In OSCC patients, does BD model have a prognostic power?" We used the reporting recommendations for tumour marker prognostic studies (REMARK) criteria to evaluate the quality of studies eligible for systematic review and meta-analysis.

Results: Nine studies were relevant as they analysed the BD model for prognostication of OSCC. These studies used either haematoxylin and eosin (HE) or pan-cytokeratin (PCK)-stained resected sections of OSCC. Our meta-analysis showed a significant association of BD model with OSCC disease-free survival (hazard ratio = 2.02; 95% confidence interval = 1.44–2.85).

Conclusions: The BD model is a simple and reliable prognostic indicator for OSCC. Evaluation of the BD model from HE- or PCK-stained sections could facilitate individualized treatment planning for OSCC patients.

KEYWORDS
BD model, depth of invasion, oral squamous cell carcinoma, prognostication, survival analysis, tumour budding

1 | INTRODUCTION

Globally, oral squamous cell carcinoma (OSCC) constitutes 4.7%–20.3% of all head and neck malignancies (Chen et al., 2016; Siegel et al., 2017) and has less than 50% 5-year overall survival rate (Kim et al., 2016). Patients with recurrent OSCC have reduced survival rates even with early-stage disease, as 20%–40% of the patients have occult cervical lymph node metastasis (Bittar et al., 2016; Safi et al., 2017).

Treatment planning in OSCC is usually based on the cTNM classification and tumour histological grade (El-Naggar et al., 2017).

However, several studies have highlighted shortcomings of the WHO histologic grade classification in predicting the prognosis of OSCC patients (Almangush et al., 2015; Brierley et al., 2017; Müller, 2017). In addition, disease progression and treatment response still differ conspicuously among patients with a similar cTNM stage (Lindenblatt et al., 2012; Low et al., 2015; Sawazaki-Calone et al., 2015). Therefore, it is crucial to identify more credible prognostic models based on histological features of the tumour to develop more appropriate treatment plans for OSCC patients. Almangush and colleagues first introduced the prognostic BD score for OSCC, which consists of the histological parameters tumour budding (B) and depth of invasion (D), (Almangush et al., 2015).

Tumour budding is defined as fewer than five cancer cells forming a cell cluster(s) at the invasive front of the tumour (Kadota et al., 2015; Rogers et al., 2016). Tumour budding has been shown to correlate with epithelial to mesenchymal transition (EMT) in many carcinomas, including tongue cancer (Liang et al., 2013; Wang et al., 2011). In addition, tumour budding is a risk factor for a late neck recurrence (Hori et al., 2017). Depth of invasion (D) is defined as a measurement from the basement membrane zone to the deepest point of cancer cell invasion (Berdugo et al., 2019). In the recent 8th edition of the American Joint Committee on Cancer (AJCC) classification, depth of invasion has been added into the TNM staging of the tumour to improve its prognostic value (Amin et al., 2017).

Many studies have reported on the prognostic value of the BD model in cancers from different subsites of the oral cavity (Almangush et al., 2015; Hori et al., 2017; Sawazaki-Calone et al., 2015; Seki et al., 2016; Strieder et al., 2017; Yu et al., 2019). These publications highlighted the future prospect of considering BD scores in treatment planning of OSCC. Our hypothesis was to validate through pooled analyses of the published studies whether BD model has a significant prognostic value in OSCC. Therefore, this study aimed to systematically review the literature to retrieve all publications related to the BD model in OSCC and to conduct a meta-analysis of the eligible studies.

2 | METHODS

2.1 | Search protocol

Two independent reviewers A.W and O.O conducted the systematic review.

The databases of Ovid MEDLINE, PubMed, Scopus, and Web of Science were searched using the following keywords: ('oral' or 'mouth' or 'tongue' or 'floor of mouth' or 'lip' or 'gingiva' or 'buccal' or 'palate') AND ('cancer' OR 'carcinoma' OR 'neoplasm' OR 'tumor' OR 'tumour') AND ('bd' OR 'bd model' OR 'budding and depth' OR 'budding and depth of invasion'). The PICO question was formulated as follows: "In OSCC patients, does BD model have a prognostic power?"

Studies in English language that evaluated BD model in cohorts of OSCCs were included in our systematic review. Studies in other languages or studies which did not present BD model analysis in OSCC were excluded. References of all relevant articles were also manually searched to ensure inclusion of all eligible studies. Studies on animal samples and conference abstracts were also excluded. The end point of the search was February 2020. RefWorks software was used to manage records and the data.

2.2 | Combination of tumour budding (B) and depth of invasion (D)

The BD model is originally based on the following three scores: 0, 1 and 2 (Almangush et al., 2015). A tumour with a depth of invasion <4 mm and fewer than five buds of carcinoma cells at the invasive front is considered low risk and scored "0." A tumour with depth of invasion ≥4 mm and with fewer than five buds at the invasive front, or a superficial tumour with <4 mm depth of invasion but with five or more buds at the invasive front is considered at intermediate risk and scored "1." A tumour with depth of invasion ≥4 mm and with five or more buds is considered high risk and scored "2" (Figure 1. Copyright (2016) Wiley. Used with permission from Strieder *et al*. Comparative

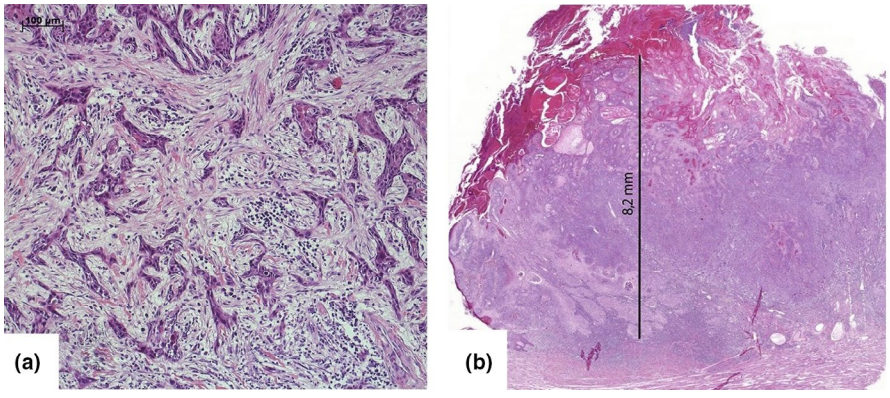

FIGURE 1 Budding and depth of invasion model: (a) Buds or islands of tumour invasion with <5 cells (100 µm). (b) Length of the tumour area with greatest depth of invasion ≥4 mm. Copyright (2016) Wiley. Used with permission from (Strieder *et al*. Comparative analysis of three histologic grading methods for squamous cell carcinoma of the lip, *Oral Diseases*, John Wiley and Sons).

analysis of three histologic grading methods for squamous cell carcinoma of the lip, *Oral Diseases*, John Wiley and Sons).

2.3 | Quality assessment

The reporting recommendations for tumour marker prognostic studies (REMARK) guidelines (Altman et al., 2012) were followed to assess the quality of studies that evaluated the prognostic value of the BD model in OSCC. The guidelines for systematic review and meta-analysis of prognostic factor studies were followed (Riley et al., 2019) along with the preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P), (Moher et al., 2015).

2.4 | Statistical methods

The statistical software R (version 3.4.0) was used to run the "meta" package (version 4.8-1) for meta-analysis. For each analysis, both an inverse variance weighted fixed-effect analysis was performed. The random-effect model analysis was considered as the main result to account for possible heterogeneity among studies (Higgins & Thompson, 2002). In addition to meta-analysed effect sizes, we also included the estimated proportion of variation in effect sizes due to heterogeneity (I^2).

3 | RESULTS

3.1 | Search results

A total of 396 hits were retrieved from databases. There were 116 duplicates that were deleted and 280 studies were included for further analyses (Figure 2). Out of these, nine studies related to the BD model in OSCC were included in this systematic review. Among these, three studies were from Japan, one from Finland, two had cases from both Finland and Brazil, two were from Brazil, and one was from China. In Table 1, each study is reported with the total number of cases, TNM stage, oral subsite, primary treatment, follow-up time, staining method/s used, cut-off point for budding and depth of invasion, survival or sensitivity and specificity analyses, hazard ratio (HR) and P value.

3.2 | Meta-analysis of disease-free survival

Only four studies reported detailed statistical analyses for disease-free survival (DFS), including HRs and 95% confidence intervals (CI); these studies were included in the meta-analysis visualized using forest plots (Figure 3a,b).

The first forest plot (Figure 3a) included all multivariate results of BD as the predictor of interest and DFS as the response variable (HR = 2.02, 95% CI = 1.44–2.85). In the second forest plot (Figure 3b), both multivariate and univariate analyses that reported HR values for BD as the predictor and DFS as the response variable were combined to attain cumulative effect size (HR = 1.82, 95% CI = 1.44–2.29). The results of the meta-analysis did not present any heterogeneity ($I^2 = 0$). Funnel plots were employed to evaluate bias across studies with HR as the effect estimate and standard error as the measure of precision. In the funnel plots (Figure 4a,b), the effect estimates were within the expected 95% region marked by the diagonal segments and neither funnel plot indicated a bias.

3.3 | Association of the BD model with disease-specific survival and overall survival

Although the published results (Table 1) on the association of BD model with disease-specific survival (DSS) and overall survival (OS) were not enough to conduct meta-analyses, there was some

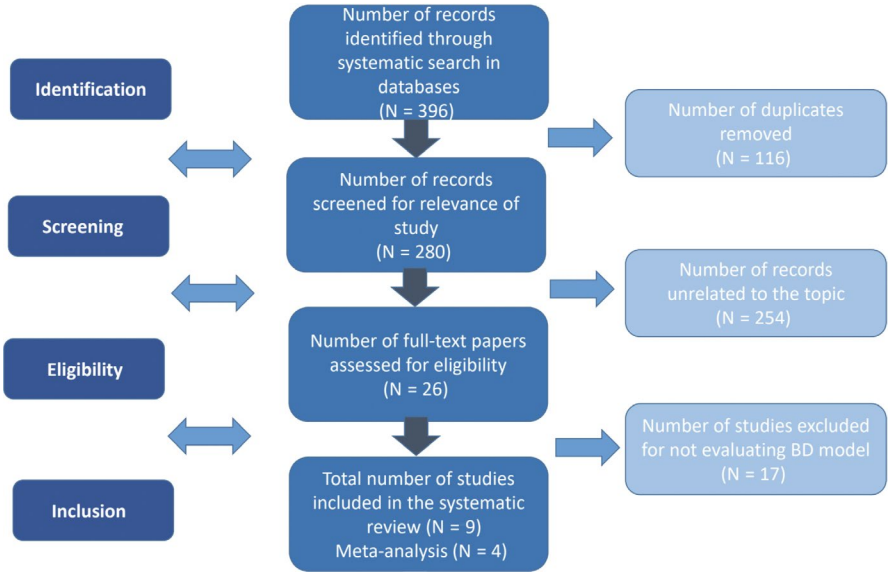

FIGURE 2 Flow diagram outlining the search strategy and the search results along various steps

TABLE 1 Summary of studies that examined the prognostic value of BD model in OSCC

Authors, year (Country)	No. of cases	TNM stage	Oral subsite	Primary treatment	Follow-up
Almangush et al. (2015) (Finland and Brazil)	311	T1–2N0	Tongue	Surgery	57 months
Sawazaki-Calone et al. (2015) (Brazil)	113	T1-T4, N0, *N+*	Oral cavity	Surgery, surgery + postoperative RT, surgery + postoperative RT + CT	5 years
Seki et al. (2016) (Japan)	91	T1-T4	Tongue and floor of mouth	Surgery, preoperative CT	4 months – 5 years
Strieder et al. (2017) (Brazil)	53	T1-T4, N0,*N+*	Lip	Surgery, surgery + RT	57.5 months (T1/T2), 159.4 months (T3/T4)
Hori et al. (2017) (Japan)	48	cT1/2 N0M0	Tongue	Surgery	71 months
Almangush, Heikkinen, et al. (2018) (Finland and Brazil)	224	T1–2N0	Tongue	Surgery	NA
Almangush, Leivo, et al. (2018) (Finland)	100	NA	Tongue	Surgery	NA
Yu et al. (2019) (China)	246	T1- T2/T3-T4	Tongue	Surgery	60 months
Hori et al. (2020) (Japan)	62	T1–2N0	Tongue	Surgery	68 months

Values in bold are from multivariate analysis.

Almangush et al., 2015 and Almangush, Heikkinen, et al., 2018 are overlapped.

HR and CI reported by Almangush, Leivo, et al., 2018 were for combined score of tumour-stroma ratio and BD model.

Hori et al., 2017 and Hori et al., 2020 are overlapped.

Abbreviations: BD, budding and depth of invasion; CI, confidence interval; CT, chemotherapy; DFS, disease-free survival; DSS, disease-specific survival; H-E, haematoxylin and eosin staining; HR, hazard ratio; HRS, histological risk score; iBD, inflammatory response, budding and invasion depth; IHC, immunohistochemical staining with cytokeratin or pan-cytokeratin; NA, not available; NPV, negative predictive value; OS, overall survival; PCK, pan-cytokeratin; PPV, positive predictive value; RT, radiotherapy.

evidence indicating a promising prognostic value for the BD model in prediction of these two survival outcomes. For example, Almangush et al. (2015) revealed a significant association of the BD model with DSS of OTSCC patients (HR = 5.11, 95% CI 2.05–12.75; $p < .001$). In contrast to other parameters WHO grading system, histological risk (HR) model and malignancy grading of the deep invasive margins (MG), only the BD model correlated with DSS ($p = .009$) in a study by Sawazaki-Calone et al. (2015). Overall survival (OS) is significantly associated with both budding and tumour depth of invasion ($p < .05$) in Seki et al. (2016). These results were similar to those observed by Strieder et al. (2017), where OS was also associated with the BD model ($p = .045$). Similarly, Yu et al. (2019) demonstrated that BD score associated with OS (HR = 2.77, 95% CI 1.78–4.33; $p < .001$).

3.4 | Association of the BD model with clinicopathological factors

A significant association ($p < .001$) of the BD model with histological risk (HR) model was demonstrated by Sawazaki-Calone et al. (2015)

Staining method	Cut-off for budding	Cut-off for depth	Survival Analysis/ Sensitivity, Specificity	HR (95% CI)	*P* value	REMARK Quality of studies
H-E	5 buds	4 mm	DSS	6.24 (2.59–15.04) **5.11 (2.05–12.75)**	< 0.001 **<0.001**	7
			DFS	2.14 (1.22–3.74) **2.19 (1.20–4.00)**	0.025 **0.033**	
H-E	5 buds	4 mm	DSS	DSS in 5 years 32%	0.009	6
			DFS	**1.93 (1.23–3.00)** DFS in 5 yrs 49%	**0.003** 0.005	
			HRS		<0.001	
IHC	>3 buds	>3 mm	OS	100% sensitivity >3 mm budding	< 0.05	6
			DFS		**<0.05**	
			Sensitivity			
H-E	5 buds	4 mm	OS (5 years)	75% (high risk cases)	0.045	6
H-E	5 buds	>3 mm	Sensitivity	89% Sensitivity		6
			Specificity	95% Specificity		
				80% PPV 97% NPV		
H-E	5 buds	4mm	DFS	**3.42 (1.71–6.82)** 2.82 (1.46–5.42)	**0.004** 0.014	8
			DSS	**11.63 (3.83–35.31)** 10.43 (3.51–31.01)	**<0.001** <0.001	
H-E	5 buds	4mm	Sensitivity	57.1% (95% CI 39.4% –73.7%)	Chi- square test	6
			Specificity	96.9% (95% CI 89.3%–99.6%)	<0.001	
H-E	5 buds	4 mm	OS	BD, 2.77 (1.78–4.33)	<0.001	8
			DFS	BD, 1.66 (1.21–2.27)	0.002	
H-E and PCK	5 buds	>3 mm	DFS	**BD, 2.06 (0.64–6.57)**	0.22	7
			Lymph node recurrence	BD, 5.46 (1.08–27.52)	<0.05	

and Seki et al. (2016), who reported 100% sensitivity for tumour budding (≥3) and tumour depth of invasion (≥3 mm) for neck metastasis (Seki et al., 2016). A strong association was also reported between neck recurrence and the BD model with 89% sensitivity and 95% specificity by Hori et al. (2017). A recent publication (Hori et al., 2020) revealed an association between neck recurrence and the BD model (HR = 5.46, 95% CI 1.08–27.52; $p < .05$). Yu et al. (2019) observed that T-stage ($p = .002$), lymph node metastasis ($p < .001$), clinical stage ($p < .001$), invasive pattern ($p < .001$), Glasgow Microenvironment Score (GMS) ($p = .031$) and tumour-to-stroma percentage ($p = .006$) were all associated significantly with BD scores.

4 | DISCUSSION

This study aimed to meta-analyse the prognostic significance of BD model in studies of OSCC. It was hypothesized that BD model

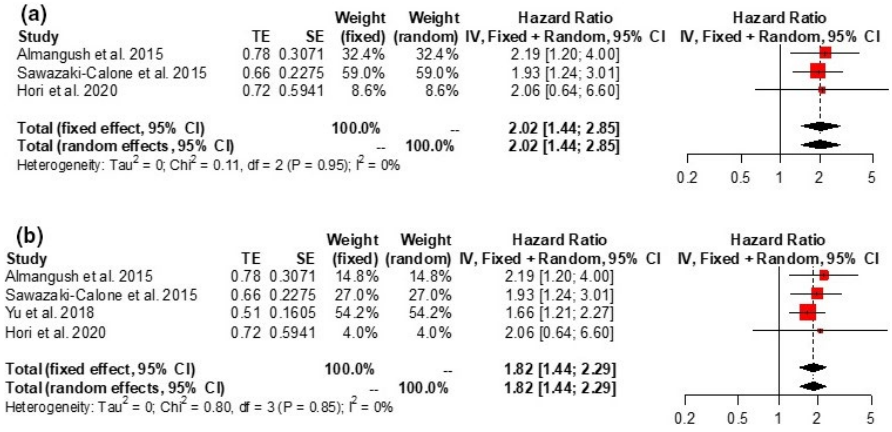

FIGURE 3 Forest plots for the pooled analyses of the studies that evaluated the prognostic value of the BD model in assessing DFS of OSCC. (a) All multivariate studies. (b) Multivariate and univariate results combined to obtain the cumulative result of meta-analysis (Few confidence limits are marginally different from the original values due to the rounding of hazard ratios and confidence limits to two digits).

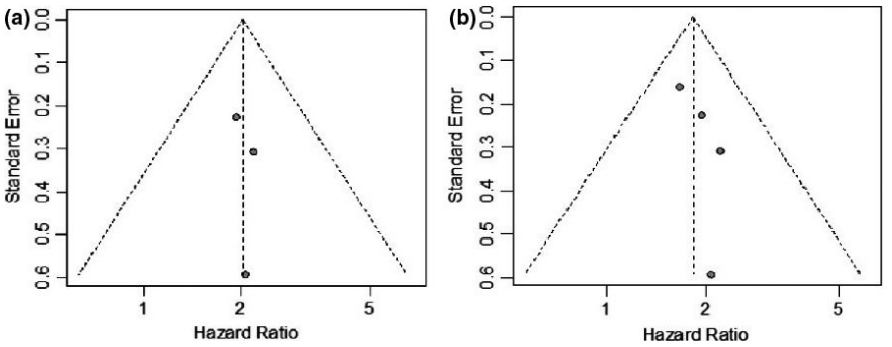

FIGURE 4 Funnel plots constructed to assess overall effect. (a) All multivariate studies, (b) all multivariate studies and one univariate study

has a prognostic role in OSCC. For this purpose, databases were searched to gather studies of BD model for a systematic review and meta-analysis.

The recent WHO classification of head and neck tumours pointed out that the conventional histological grading (well, moderate or poorly differentiated OSCC) correlates poorly with clinical outcome, and a non-cohesive pattern of invasion, perineural and lymphovascular invasion, bone invasion and tumour thickness of ≥4 mm is associated with poorer prognosis (El-Naggar et al., 2017). The histological grading system is easier to analyse and is therefore still widely used in clinical pathology reports. However, this system has been widely criticized by several authors (Brierley et al., 2017; Müller, 2017). Here, our meta-analysis revealed that readily available BD score is significantly associated with DFS as an independent prognostic indicator. Additionally, in many studies a number of clinicopathological factors (such as T-stage, lymph node metastasis, clinical stage, invasive pattern) and both of OS and DSS of OSCC patients were significantly associated with the BD model.

In our systematic review, all the studies used surgery as primary treatment. Some studies presented significant results of the BD model for OSCC in OS: (HR = 2.77, 95% CI 1.78–4.33; $p < .001$), (Yu et al., 2019), and DSS: (HR = 5.11, 95% CI 2.05–12.75; $p < .001$) (Almangush et al., 2015; Yu et al., 2019). Specificity of 93% and 96.9% for BD model was presented by Hori et al. (2017) and Almangush, Leivo, et al. (2018), respectively. However, the published studies were insufficient to conduct meta-analyses on OS or DSS. Four original studies had appropriate statistics for meta-analyses of DFS. To avoid bias due to potential heterogeneity, a random-effect model (in addition to a fixed-effect model) was employed to combine heterogenous studies (Guolo & Varin, 2015). However, as we did not detect any heterogeneity, therefore the random-effect model produced the same results as the fixed-effect model. We conducted two meta-analyses (Figure 3), one with HR values from the three multivariate analyses for DFS (Almangush et al., 2015; Hori et al., 2020; Sawazaki-Calone et al., 2015) (Figure 3a), and the other that also included one additional study (Yu et al., 2019) that provided only univariate analysis results for DFS (Figure 3b). Both forest plots indicated significant meta-analysed HR values and thus validated the results of the studies that presented prognostic results of the BD model in DFS of OSCC patients. Based on these analyses, the BD model was shown to be a prognostic indicator for DFS in OSCC, which was consistent across these studies. Of note, tongue was the oral subsite in most of the included studies. However, inclusion of different subsites along with different stages in the meta-analysis was reported and is among the limitations. Despite these shortcomings, the gathered evidence was sufficient to propose that a high BD score is an indicator for poor prognosis in OSCC.

In the literature, tumour budding has been analysed in at least 10 studies of OSCC (Almangush et al., 2015; Angadi et al., 2015; Arora et al., 2017; Box berg et al., 2017; Hori et al., 2017; Jensen et al., 2015; Manjula et al., 2015; Pedersen et al., 2017; Seki et al., 2016; Xie et al., 2015). The cut-off point of five buds was advocated in most of these studies, and only one group (Seki et al., 2016) adjusted the cut-off point of three buds. Budding represents two malignant features, namely discohesion of cells and active invasion. Therefore, tumour budding has been considered as characteristic

behaviour of an aggressive tumour (Wang et al., 2011). Recently, a study presented tumour budding and worst pattern of invasion to be important risk factors to predict lymph node metastasis in all stages of OSCC (Chatterjee et al., 2019). Outside the oral cavity, tumour budding is associated with poor prognosis in several other malignancies, such as bladder, cervical, rectal, breast, cutaneous and hepatocellular cancers (Fukumoto et al., 2016; Huang et al., 2016; Jäger et al., 2018; Lino-Silva et al., 2018; Voutsadakis, 2018; Wei et al., 2020).

Depth of invasion has predictive value for lymph node metastasis and loco-regional recurrence in OSCC (Shinn et al., 2018). In our review, six of the studies set a cut-off point of 4 mm (Almangush et al., 2015; Almangush, Heikkinen, et al., 2018; Almangush, Leivo, et al., 2018; Sawazaki-Calone et al., 2015; Strieder et al., 2017; Yu et al., 2019), and in three Japanese studies (Hori et al., 2017, 2020; Seki et al., 2016), the cut-off point for depth of invasion was adjusted at 3 mm. The latest cancer staging manual of the AJCC (8th edition) considers depth of invasion in the TNM classification system (Amin et al., 2017). This addition into the TNM classification already improves the predictive value and stratification of different TNM stages, including recognition of low-risk patients with a reduced survival rate (Rodrigues et al., 2020).

The BD score is relatively simple for objective analysis (Sawazaki-Calone et al., 2015), and as it is possible to evaluate even from the originally HE-stained slides, the analyses are inexpensive and the score can easily be added to clinical pathology reports. In contrast, the other histological models, such as malignancy grading of the deep invasive margins (MG), (Bryne et al., 1992) and histological risk (HR) model (Brandwein-Gensler et al., 2005), are more difficult to apply (Bundgaard et al., 2002; Gueiros et al., 2011; Lindenblatt et al., 2012). To the best our knowledge, this is the first systematic review and meta-analysis on the prognostic value of BD model in OSCC.

5 | CONCLUSION

In this systematic review, we found a total of nine studies that all presented significance of BD model as a prognosticator in OSCC. Based on the current meta-analysis, we here conclude that the BD model has a strong prognostic power for DFS in OSCC patients. In the future, other practical histopathological models, such as tumour-to-stroma ratio and tumour infiltration lymphocytes, combined with BD score, should be tested to possibly upgrade the prognostic power of histopathological features in OSCC.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTION

Awais Wahab: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Writing-original draft; Writing-review & editing. **Oona Onkamo:** Conceptualization; Data curation; Formal analysis; Methodology; Writing-original draft; Writing-review & editing. **Matti Pirinen:** Conceptualization; Formal analysis; Investigation; Validation; Visualization; Writing-original draft; Writing-review & editing. **Alhadi Almangush:** Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Supervision; Validation; Visualization; Writing-original draft; Writing-review & editing. **Tuula Salo:** Conceptualization; Funding acquisition; Methodology; Resources; Supervision; Validation; Visualization; Writing-original draft; Writing-review & editing.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/odi.13671.

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